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Joint toxicity of alkoxyethanol mixtures: Contribution of *in silico* applications☆

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Abstract

Exposure to chemicals occurs often as mixtures. Presented in this paper is information on alkoxyethanols and the impact they might have on human health in combination with some commonly found aliphatic and aromatic compounds. Our studies to evaluate the joint toxicity of these chemicals among themselves and in combination with other chemicals reveal a variety of possible outcomes depending on the exposure scenario. The interactions are predominantly based on metabolic pathways and are common among several solvents and organic compounds. Quantitative structure activity relationship (QSAR) analysis can be used with high confidence to identify chemicals that will interact to influence overall joint toxicity. Potential human exposure to a combination of alkoxyethanol, toluene and substituted benzenes may increase reproductive and developmental disease conditions. Inheritable gene alterations result in changes in the enzyme function in different subpopulations causing variations in quantity and/or quality of particular isoenzymes. These changes are responsible for differential metabolism of chemicals in species, genders, and life stages and are often the basis of a population's susceptibility. Unique genotypes introduced as a function of migration can alter the genetic makeup of any given population. Hence special consideration should be given to susceptible populations while conducting chemical health risk assessments.

Keywords

Alkoxyethanols; Alcohol dehydrogenase; Aldehyde dehydrogenase; Mixtures toxicity; QSAR modeling; Genetic variability

1. Introduction

Alkoxyethanols are widely used in industrialized countries as solvents in spray lacquers, enamels, varnishes, latex paints, as ingredients in paint thinners/strippers, varnish removers, and in herbicide formulations. Alkoxyethanols can be represented by two distinct toxicophore areas (Fig. 1): (a) R1 representing the alkyl ether group (the methoxy-, ethoxy-,

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propoxy-, or butoxy-group), and (b) R2 representing the ethylacetate and alcohol groups and their metabolites (acetaldehyde, acetic acid).

Their use and health effects have recently attracted the attention of international organizations such as the World Health Organization (WHO) (CICAD, 2011). Human exposure to these chemicals usually occurs through dermal and inhalation routes. Limited data indicate that these chemicals are readily absorbed. The most studied members of this family viz., 2-methoxy-, 2-ethoxy-, 2-propoxy-, and 2-butoxyethanol cause moderate acute toxicity in laboratory animals that includes lethargy and inactivity. High doses may lead to narcosis and death. These symptoms are indicative of non-specific depression of the central nervous system typical of organic solvents.

As is true in many chemical families, alkoxyethanols as a group, have similar toxicity. They show a dose–response relationship, and their toxicity decreases as a function of the length of the alkyl ether chain (Nagano et al., 1979; Katz et al., 1984). Some exposure route dependent differences have been documented. For example, 2-butoxyethanol appears to be more toxic by the inhalation and dermal routes than the other members of the group. Long term exposures to these chemicals are associated with specific effects such as reproductive, hematological, and developmental toxicity. Attempts have been made to elucidate the mode of action of these chemicals. For example, the embryo-toxicity is believed to be caused by decreased intracellular pH induced by the metabolite – alkoxyacetic acid (Louisse et al., 2010). *In vitro* exposure caused acidification of murine embryonic fibroblast cells (BALB/c 3T3) and resulted in inhibition of murine stem cells (ES-D3) differentiation. Hematotoxicity was attributed to butoxyacetic acid-induced hemolysis of rat red blood cells following *in vitro* exposure to ethylene glycol monobutyl ether (Udden and Patton, 2005). The hemolysis was caused by changes in external osmolarity and cation levels.

Metabolism of alkoxyethanols is via a pathway involving oxidation by alcohol dehydrogenase (ADH) enzymes to an intermediate alkoxyacetaldehyde, followed by quick conversion by aldehyde dehydrogenases (ALDH) to the corresponding alkoxy acetic acid (Foster et al., 1984) (Fig. 2). The latter metabolite is considered in each case to be the major cause of observed toxicity. The alkoxyacetic acid subsequently may be conjugated with glycine or be *O*-dealkylated and further metabolised to yield carbon dioxide. A secondary pathway for metabolism of the alkoxyethanols involves microsomal P-450 mixed-function oxidases, with dealkylation generating ethylene glycol. Direct conjugation with sulphate or glucuronic acid may also occur. The principal route of elimination for the alkoxyethanols is via the urine as the alkoxyacetic acid metabolite.

Distinct differences in the metabolism of the ethylene glycol ethers as a function of alkyl ethers chain length were reported following oral exposure in rats (Medinsky et al., 1990). Alkoxyethanols with a short alkyl ether chain are likely to have a greater portion of the dose metabolized via the pathway involving microsomal P450 mixed function oxidases with the production of ethylene glycol. Alkoxyethanols with longer alkyl ether chains preferentially use the pathway resulting in formation of acetic acid derivatives. For example, 34% of 2-methoxyethanol was eliminated in urine as methoxyacetic acid and 21% as ethylene glycol. In contrast, 50–60% of 2-butoxyethanol was eliminated as butoxyacetic acid and only about

10% as ethylene glycol. Ethylene glycol pathway was described as alternative and less toxic (Medinsky et al., 1990).

However, alcohol dehydrogenase and aldehyde dehydrogenase play important roles in metabolism of all alkoxyethanols. Competitive inhibition in the joint toxic action of alkoxyethanols may result in altered toxicity.

Exposure to most chemicals occurs as mixtures, and alkoxyethanols are no exceptions. The purpose of this paper is to evaluate the influence of interactions on the joint toxicity of alkoxyethanols using a weight-of-evidence method, and to identify chemicals, based on quantitative structure activity relationship (QSAR) analysis, that have a potential to interact with alkoxyethanols. In addition, this paper reviews genetic polymorphisms of the key enzymes and any impact of their variability on risk assessment, particularly in sensitive populations.

2. Methods

2.1. Mixtures evaluation

There are several options available to assess the joint toxicity of chemical mixtures (ATSDR, 2004; USEPA, 2000). These assessments can be based on literature data available for the mixture of concern (whole mixture), on a similar mixture, or on mixture's components. In the absence of whole mixture data the component based approaches are used and these include the hazard index (HI) method, the target-organ toxicity dose (TTD) modification to the HI method, weight-of-evidence (WOE) modification to the HI method, toxic equivalency (TEQ) and relative potency, and computational methods such as physiologically based pharmacokinetic/pharmaco-dynamic (PBPK/PD) and QSAR modeling (ATSDR, 2004).

2.2. Hazard Index

The hazard index (HI) approach uses the assumption of potency weighted additivity (dose or response) to assess the joint toxicity of a mixture from the data on the components. The approach is used or recommended by a number of agencies (ACGIH, 2000; EPA, 1986, 1989a, 1990; National Academy of Sciences [NAS], 1974; National Research Council [NRC], 1989; OSHA, 1993, 2001). Exposures or doses for the various components of the mixture are scaled by a defined level of exposure generally regarded as "acceptable" or "safe" by the agency performing the assessment. The defined levels could be ATSDR minimal risk levels (MRLs), EPA reference doses (RfDs) or reference concentrations (RfCs), ACGIH threshold limit values (TLVs), or OSHA permissible exposure limits (PELs). The advantages and limitations of HI approach are discussed in the above cited references.

2.3. The weight-of-evidence (WOE) approach

Component-based approaches such as the hazard index approach are most useful when augmented with a weight-of-evidence evaluation of the potential for nonadditive interactions among the components in the mixture. The WOE approach for assessing toxicological interactions in chemical mixtures has been developed by Mumtaz and Durkin (1992) and

further explained by Mumtaz et al. (1994). In this approach, a mixture is broken down into binary pairs and a determination is made on the interaction potential of each pair of chemicals. This determination is called a binary weight-of-evidence determination (BINWOE). A BINWOE is based on empirical observations and mechanistic considerations, that categorizes the most plausible nature of any potential influence of one compound on the toxicity of another compound for a given exposure scenario (Table 1). These interaction determinations are based on an evaluation of the available information on the metabolism, health effects and other pertinent data available in the literature on the chemicals. First, the direction of interaction is predicted (greater than additive, less than additive, or additive), and then a classification is assigned on the basis of the mechanistic understanding and toxicological significance of the interaction determination. If the component-based analyses indicate that several binary combinations will have more than additive joint toxicity, the HI could underestimate the final toxicity and vice versa.

2.4. QSAR identification of chemicals that have a potential for developmental toxicity (DT)

TOPKAT 6.2, a commercially available software, was used for quantitative structure activity relationship (QSAR) analysis to identify chemicals that could potentially interact. This software consists of a variety of models including developmental effects. Each of TOPKAT's QSAR model can be represented as:

$$T=\sum_{i=1}^{n}\beta_i X_i+C$$

where T is the predicted toxicity score, C is a constant, X_i is the ith structure descriptor value, and β_i is the coefficient associated with the descriptor. The product $\beta_i X_i$ represents the contribution of the ith descriptor to the predicted toxicity value, which is the sum of descriptor contributions and the constant C. For a classification model making a dichotomous prediction, this sum is transformed into a probability value between 0 and 1 for the positive class (e.g., mutagen, carcinogen). Toxicity predicted with probability values between 0 and 0.3 are considered negative or of low probability of being positive, while those between 0.3 and 0.7 are considered indeterminate (i.e., no unequivocal prediction). A positive prediction is indicated when the probability is greater than 0.7. The details of the assessment process using the TOPKAT software have been previously published (Moudgal et al., 2000; Ruiz et al., 2008; Pohl et al., 2010; Ruiz et al., 2011).

The DT model of this software consists of three submodels; each developed from a specific class of chemicals, aliphatic, hetero-aromatic and carbo-aromatic (Gombar et al., 1995). These models were derived from 374 uniform experimental studies selected after a critical review of approximately 3,000 open literature citations. They compute the probability that a query structure will exhibit developmental toxicity in the rat when tested up to a dose inducing maternal toxicity. The performance of the developmental toxicity module in the leave-one-out cross-validation study is shown in Table 2. Since leave-one-out is the least stringent of the cross-validation techniques, one may get significantly lower sensitivity, specificity, and forecast accuracy when this model is applied to new test sets.

Chemicals such as xylene and toluene have been shown to interact with ethoxyethanols at the enzymatic level and influence their toxicity outcome, particularly testicular toxicity. A unique feature of TOPKAT, called “QSAR similarity search” was used to identify chemicals that could cause developmental toxicity and are structurally similar to xylene and toluene. Unlike other similarity measures, QSAR similarity search, expressed as the Euclidian distance computed from the values of model descriptors, is property sensitive because it reflects the similarity of descriptor values between two molecules with respect to a specific property or endpoint (Accelrys, 2004). When implemented this type of search mines the associated database of a QSAR model to look for the most similar compounds and assigns surrogate chemicals and prediction confidence based on the similarity distance and concordance between the experimental and predicted values of these similar compounds (Gombar, 1997; Moudgal et al., 2003; Venkatapathy et al., 2009). The flow chart in Fig. 3 shows a decision tree used to assign confidence in the model prediction (Ruiz et al., 2011). To assign high, moderate, or low confidence in a prediction the nearest four neighbors (chemicals 1, 2, 3, and 4) in the data base with a similarity distance of <0.25 are considered. For example, if 3, 4 of the nearest chemicals found in the database at a similarity distance <0.25 are predicted accurately, the confidence is assigned as high.

2.5. Health risk assessment and genetic polymorphism

Using Japanese volunteers, Mizoi et al. (1994) evaluated the role of genetic variations in ADH and ALDH 2 in ethanol metabolism. The investigators administered 0.4 g of ethanol per kg of body weight over 10 min to 68 healthy subjects. Although the authors did not find significant difference among ADH genotypes, the acetaldehyde levels were significantly different by ALDH2 genotype. The subjects carrying the ALDH2*1/*2 genotype (43%) had blood acetaldehyde levels of 23.4 μM on average. Subjects homozygous for ALDH2*2 (9%) showed very high levels of blood acetaldehyde with average value of 79.3 μM, whereas the average value of blood acetaldehyde was 4.1 μM for subject homozygous (48%) to the wild type variant (ALDH2*1/*1 genotype). Therefore, in Japanese individuals with the homozygous variant blood acetaldehyde levels were 20 times higher than individuals with the homozygous wild type. Due to reduced clearance of acetaldehyde in homozygous ALDH2*2 subjects, their levels of acetic acid are expected to be lower than corresponding levels in homozygous wild-type individuals. Therefore, this subpopulation may be at greater risk of effects associated with acetaldehyde metabolites, but at lower risk of effects associated with acetic acid metabolites, when compared to homozygous wild-type individuals. In this case, the default toxicokinetic uncertainty factor of (3.16) may not provide adequate protection in homozygous ALDH2*2 individuals from potential effects associated acetaldehyde metabolites. Using the data of Mizoi et al. (1994) in combination with the estimated frequencies of the ALDH2 genotype in the US population until 2010, we have generated a Monte Carlo population distribution of blood acetaldehyde levels after alcohol intake. The genotype frequencies used to estimate the ALDH2 genotypic distribution in the total US population are 4.07% for ALDH2*2/*2 genotype for Asian individuals, 0% for non-Hispanic white and Mexican–American and other race/ethnicity. The frequencies of ALDH2*1/*2 genotype are 26.6% for Asian individuals, 0.65% for non-Hispanic white and Mexican–American individuals. The frequencies of ALDH2 *1/*1 geneotype are 69.3% for Asian individuals, 99.3% for non-Hispanic white and Mexican–American and100% for

persons from other race/ethnicities (Scinicariello and De Rosa, 2007). The Monte Carlo simulation was performed using the ranuni and rannor functions with 50,000 iterations in SAS 9.2 (Fan et al., 2003). The methodology is used here to make regional projections, as well.

3. Results and discussion

3.1. Mixtures evaluation – WOE predictions on interactions

Additive joint toxicity is expected following co-exposure to alkoxyethanols at low dose levels and less than additive at high dose levels due to competitive inhibition, primarily at the ADH level. This hypothesis was confirmed in laboratory animals when competitive inhibition of ADH increased individual LD_{50} of 2-methoxyethanol and 2-ethoxyethanol during co-exposure [i.e., toxicity was decreased] (Bonitenko et al., 1990). It was demonstrated that pyrazole, a known inhibitor of alcohol dehydrogenase, provided complete protection against testicular damage otherwise induced by 2-methoxyethanol or 2-ethoxyethanol (Foster et al., 1984). Similarly, pyrazole prevented the metabolism of 2-butoxyethanol to 2-butoxyaldehyde and eventually to 2-butoxyacetic acid thus protecting rats against 2-butoxyethanol-induced hematotoxicity observed in the experimental group treated with the alkoxyethanol alone (Ghanayem et al., 1987). Further, pretreatment with cyanamide, a known inhibitor of ALDH, protected rats against 2-butoxyethanol-induced hematotoxicity by preventing conversion to the ultimate toxic metabolite 2-butoxyacetic acid. In both experiments, increased levels of glucuronate and sulfate conjugates of 2-butoxyethanol indicated changes in metabolism. The study demonstrated a good correlation between the 2-butoxyethanol-induced hematotoxicity and the amount of free 2-butoxyacetic acid in urine. Therefore, competitive inhibition at the ADH or ALDH levels may change the toxicity outcome.

Based on the foregoing studies, we propose that competitive inhibition may also lead to decreased target organ toxicity that is otherwise induced by the respective alkoxyethanols. This is also applicable, for example, to joint reproductive toxicity caused by mixtures of 2-methoxy- and 2-ethoxyethanol at high exposure levels. Further, although 2-propoxyethanol may not show reproductive toxicity (Nagano et al. 1984; Katz 1987), it would influence the reproductive toxicity of 2-methoxy and 2-ethoxyethanol at high doses through the mechanism of competitive inhibition. In contrast, it is assumed that the mechanism of joint toxic action involving competitive inhibition is applicable to all alkoxyethanols that exhibit hematotoxicity.

Similarly, high-dose competitive inhibition at the ADH level is expected in mixtures of alkoxyethanols with other chemicals that use this enzyme in their metabolic pathways. For example, competitive inhibition is expected for the interaction of alkoxyethanols and ethanol. Both chemicals share ADH and ALDH in the respective metabolic pathways. A study in rats showed that co-exposure to ethanol and 2-butoxyethanol caused increased blood levels and prolonged duration of elimination of 2-butoxyethanol than exposure to 2-butoxyethanol alone (Romer et al., 1985). The authors attributed this result to the possible competition of both chemicals for ADH. Following this report, simulations of human pharmacokinetics were run to model co-exposure to ethanol (0.1% in blood) and 20 ppm 2-

butoxyethanol during an 8-h work period (Johanson, 1986, Johanson and Johnsson, 1991; Johanson and Naslund, 1988). The models predicted increased blood levels of 2-butoxyethanol as a result of decreased elimination. If both chemicals were to compete for the same enzyme at high exposure levels, decreased toxicity due to competitive inhibition would be expected. In fact, it was suggested to use ethanol as an antidote in alkoxyethanols poisoning (Romer et al., 1985).

Another example of competitive inhibition is the interaction of alkoxyethanols and toluene and/or xylene. A study in rats examined testicular toxicity of 2-ethoxyethanol alone and in combination with toluene and xylene (Chung et al., 1999). 2-Ethoxyethanol – induced testicular atrophy was reduced 30% by co-exposure to toluene and xylene. The mechanistic understanding of the interaction assumes that xylene and toluene compete with 2-ethoxyethanol for ADH. The competition reduces levels of 2-ethoxyaldehyde and, ultimately, 2-ethoxyacetic acid, the toxic metabolites responsible for testicular damage.

Better insight into these interactions can be obtained by visualizing the scheme for toluene metabolism. The main metabolic pathway of toluene consists of its conversion to benzyl alcohol (catalyzed mainly by CYP2E1 enzymes), further to benzoic acid (catalyzed by alcohol and aldehyde dehydrogenases), and to the final product hippuric acid (Angerer et al., 1998; Nakajima and Wang, 1994; Nakajima et al., 1997). In fact, about 75–80% of inhaled toluene that is absorbed can be detected as its principal metabolite, hippuric acid, in urine (Löf et al., 1993; Tardif et al., 1998). A competitive inhibition between toluene and xylene can be observed at high doses at the CYP2E1 level of their metabolism (Pohl and Scinicariello, 2011). However, the first opportunity for competitive inhibition between these chemicals and alkoxyethanols is at the ADH level.

Dichlorobenzene is metabolized to form active epoxide via mostly CYP2E1 enzymes. The epoxide can be conjugated to glutathione or glucuronic acid or be further hydrolyzed to form dichlorophenols. The dichlorophenol metabolites (reaction assisted by alcohol dehydrogenase) can again be conjugated with glutathione, glucuronic acid, or sulfate, or further converted to catechols (den Besten et al., 1992). Interactions with alkoxyethanols can be expected on the aldehyde dehydrogenase level.

3.2. QSAR identification of chemicals-predictions of similarity

QSAR was used to identify chemicals that may competitively interact with alkoxyethanols. A QSAR developmental toxicity (DT) analysis revealed a probability of 0.004 for toluene and a probability of 0.013 for *o*-xylene. Both the univariate and multivariate, analyses predicted each of the chemicals to be a non-developmental toxicant (negative). To identify potential surrogate chemicals for toluene and *o*-xylene in the above experimental mixture of concern (ethoxyethanol (EE), toluene and *o*-xylene) a QSAR similarity search on the complete model database was performed.

The QSAR similarity search analysis shows nine chemicals within 0.25 normalized distance of toluene in the DT model chemical space. Four of them within 0.106 vicinity of toluene are shown in Fig. 4. These chemicals are chlorobenzene, nitrobenzene, 1,2-dichlorobenzene, and diphenyl. Of the nine database chemicals within the 0.25 vicinity, 8 are non-

developmental toxicants and one is a developmental toxicant in rodents (Table 3). The DT model correctly predicted the lack of DT for eight non-DT database chemicals (NEG in Table 3). For the one remaining chemical the DT model prediction was indefinite (IND). All nine chemicals were included in the training set of the model. Thus, 88% of database chemicals within the 0.25 distance of toluene and 100% of chemicals within the 0.1 distance were correctly predicted by the model. According to the decision-matrix flowchart (Fig. 3), these results show a good agreement with the experimental data (Ruiz et al., 2011).

For *o*-xylene, the similarity search showed thirteen chemicals within 0.25 normalized distance in the DT model chemical space. Four of them within 0.104 vicinity of *o*-xylene are shown in Fig. 5. These chemicals are 1,2-dichlorobenzene, diphenyl, 2-methylresorcinol and chlorobenzene. Of the thirteen database chemicals within the 0.25 vicinity, 10 are non-developmental toxicants and three developmental toxicants in rodents (Table 4). The model was correctly able to predict the lack of DT for 10 non-DT database chemicals (negative in Table 4). For the three remaining chemicals, the model predictions were two negative and one indefinite. All thirteen chemicals were included in the training set of the DT model. Thus, 77% of database chemicals within the 0.25 distance of *o*-xylene and 100% of chemicals within the 0.1 distance were correctly predicted by the model. According to the decision-matrix flowchart (Fig. 3), these results show that the confidence in the prediction is high and is in agreement with the experimental data (Ruiz et al., 2011).

3.3. Health risk assessment and genetic polymorphism

Alcohol dehydrogenases (ADH) facilitate the conversion between alcohols and aldehydes or ketones. It is a redox reaction that involves the coenzyme nicotinamide adenine dinucleotide (NAD^+). There are seven known genes that are organized into five classes based on amino acid sequencing, catalytic properties and tissue-specific expression. Each enzyme is a dimer (i.e., consists of two polypeptides) and each dimer includes two zinc ions (one at the catalytic site and one at the structural site to stabilize the protein) (Brandt et al., 2009; Hammes-Schiffer and Benkovic, 2006). Isozymic forms exist in some of the individual classes. For example, *ADH1B* is represented by genotypes *ADH1B**1, *ADH1B**3 *ADH1B**2, are and the and enzyme subunits called β_1, β_2, and β_3. They differ in their ethanol catalytic efficiency. The β_1 allele is found predominantly in Caucasians and African–Americans, β_2 in the Japanese and Chinese, and β_3 in about 25% of African–Americans (Eckardt et al., 1998; Yin and Li, 1989). ADH activity differs in a population not only according to the genotype, but also to age and other variables. In a cohort of 111 Caucasian individuals, ADH activity decreased with increasing age in men, while ADH activity increased with age in women (Parlesak et al., 2002). In men between 20 and 40 years of age, intake of larger amounts of alcohol (>0.8 g/kg body weight/day) was associated with decreased ADH activity. Increased ADH activity can increase the rate of alcohol metabolism and results in increased production of acetaldehyde. If acetaldehyde is not metabolized fast enough, a “flushing reaction” may occur. Typical signs include facial flushing, headache, dizziness, anxiety, nausea, vomiting, decreased blood pressure, tachycardia, and severe cardiovascular problems. These adverse effects of acetaldehyde can limit alcohol consumption and, as proposed, decrease alcohol dependence (Thomasson et al., 1995; Hasin et al., 2002).

Another enzyme, aldehyde dehydrogenase (ALDH), facilitates the conversion of an aldehyde to its corresponding acid. The reaction uses $NADP^+$ as a cofactor. In mammals, there are three different classes of this enzyme. ALDH1 and ALDH2 are the most important for aldehyde oxidation. Both are tetrameric enzymes with four subunits. The *ALDH2* gene is polymorphic, and the mutant allele *ALDH2**2 produces an inactive subunit. As a consequence of this mutagenic deficiency, unmetabolized acetaldehyde accumulates in the body. Distribution of the *ALDH2**2 alleles varies by ethnicity: almost all Caucasians carry the functional *ALDH2**1/*1 genotype. The *ALDH2**2 allele is frequent among the East Asian populations, which are typically 30% ALDH2*1/2 heterozygous and 5–10% ALDH2*2 homozygous (Goedde et al., 1992). A study in Japan reported that 41% individuals in a non-alcoholic cohort were ALDH2 deficient in contrast to only 2–5% in a cohort of alcoholics.

When deriving health-based guidance values for toxic chemicals, uncertainty factors (UFs) are commonly used (Pohl and Abadin, 1995). The variability of metabolism of toxic chemicals caused by genetic polymorphisms is expected to be captured by the 3.16-fold toxicokinetic uncertainty factor (IPCS, 1994).

The contribution of the genotype frequencies of Asian-origin individuals to the total US. population is steadily captured in the upper 99th percentile of the distribution of blood acetaldehyde levels. The US Census conducted in 2010 showed that the people of Asian origin alone represented 4.8% of the population (US Census, 2010). This relatively small contribution in the total US population did not alter significantly the mean distribution, the median, and the 95th of blood acetaldehyde levels when compared to the same distribution in the population carrying the wild-type genotype ALDH2*1/*1. However, there has been a slight increase in the blood acetaldehyde inter-individual toxicokinetics, which is taken as the ratio between the 99th percentile and the median value of the blood acetaldehyde in the population (Table 5). Although these ratios are lower than the 3.16-fold default UF representing toxicokinetics, based on US Census population projections, the ratio will become 3.23, slightly higher than the 3.16-fold UF default, in 2050 (US Census, 2008).

Since the distribution of people of Asian origins varies by state, for states with higher Asian populations, this effect may even be greater. In California, the Asian population has increased from 9.8% in 1990 to 13% according to the 2010 Census. Consequently, there has been a steady increase in the 99th/median blood acetaldehyde inter-individual toxicokinetics. The calculated ratio was 5.37-, 5.71- to 6.34-fold for the 1990, 2000 and 2010. In the last 20 years, the Asian population in the states of New Jersey has doubled reaching 8.3% from 3.5%. Monte Carlo simulation showed that in New Jersey the calculated 99th/median ratio was 1.83-, 1.90- to 3.86-fold for the 1990, 2000 and 2010 Census population, respectively, with the latter ratio higher than the default for toxicokinetics in the composite UF for inter-individual variability. From 1990 to 2010, the Asian population in the states of New Jersey and New York has doubled reaching 8.3% and 7.3% in 2010, respectively. Consequently, there has been a steady increase in the blood acetaldehyde inter-individual toxicokinetics, which is taken as the ratio between the 99th percentile and the median of the population among the California, New Jersey, and New York State populations in the last 20 years. Although the estimated ratios for the New York populations are still lower than the default

toxicokinetic uncertainty factor of 3.16-fold (estimated 99th/median ratio based on 2010 US Census population is 2.13), if the migration trend is maintained, most likely the ratio will be higher that the default factor in less than a decade. The state of Hawaii has experienced a steady decrease in percentage of people of Asian origin, even though the 99th/median ratio is much higher than the default inter-individual UF tokicokinetics (Table 5).

This exercise shows how migration, both from internal or external movements, may influence the dynamic of risk assessment and that risk assessments should be continually revised. Furthermore, the value of this exercise is not confined to ethanol ingestion and metabolism; it may be expanded to other acetaldehyde-producing compounds such alkoxyethanols, ethyl acetate, and vinyl acetate. Acetaldehyde has been classified as probable human carcinogen based on chronic inhalation exposure experiment resulting in increased incidence of nasal and laryngeal tumors in rats and hamsters, respectively (US EPA, 2006). Potential carcinogenicity mode of action of acetaldehyde is through production of DNA adducts, DNA–protein cross-link and cytogenetic damage. This is reflected by the observation that *ALDH2**2 allele is also associated with increased odds for oropharyngolaryngeal, esophageal, gastric and colon cancer (Yokoyama et al., 1998).

4. Conclusion

We live in a world surrounded by chemicals and their mixtures. It is important we understand their pros and cons so that we can take advantage of their characteristics. Chemical risk assessment is a data intensive process that helps manage unexpected outcomes. We used alkoxyethanols, a group of commonly used chemicals, to illustrate the considerations that should go into the risk assessment process. First, we have shown that the joint toxicity of alkoxyethanols in combination with themselves and with other chemicals can cause a variety of possible outcomes depending on the exposure scenario. Mostly, their joint toxicity is influenced because of shared metabolic pathways with other solvents and organic compounds.

The knowledge gained from experimental testing has been applied to develop computational tools such as QSAR/SAR and other *in silico* methods that support reducing, refining, and replacing animals in toxicological testing. It is also shown, if data are lacking, that QSAR analysis can be used with high confidence to predict certain toxicity endpoints of alkoxyethanols. This type of analysis also supports the current thinking encouraging the use of alternative methods for toxicity testing to minimize potential animal suffering (NAS, 2007; Dix et al., 2007).

Another important aspect of risk assessment regarding susceptible subpopulations has also been highlighted in this paper. Inheritable gene alterations result in changes of enzyme function in different subpopulations causing variations in quantity and/or quality of particular isoenzymes. These changes are responsible for differential metabolism of chemicals in species, genders, and life stages and often are the basis of a population’s susceptibility. Some of these variations are introduced as a function of human migration. Hence, special consideration should be given to sensitive and susceptible populations while conducting chemical health risk assessments.

The socioeconomic cost of traditional toxicology can be enormous. For the past two decades, a significant effort has been dedicated to developing alternatives to laboratory testing, including computational toxicology methods in the areas of hazard identification, toxicity evaluation, and risk characterization. Many refined and validated models exist for single chemicals and, combined with traditional methods, they help to improve the risk assessment process. In this paper, we wanted to demonstrate the importance of an *in silico* method as a tool that can supplement traditional approach to risk assessment of chemical mixtures. In addition, identification of susceptibility genes provides an opportunity to protect sensitive individuals and populations based on scientific knowledge as opposed to application of traditional generic default uncertainty factors for inter-individual differences. Introducing these concepts into evaluations of joint toxicity of chemicals is a vital step forward in our approach to mixtures. Future risk assessments will become more meaningful and greatly contribute to the health of the environment and human populations as we increase our predictive capabilities and integrate the in-put of computational methods into the overall recommendations of the chemical risk assessment process.

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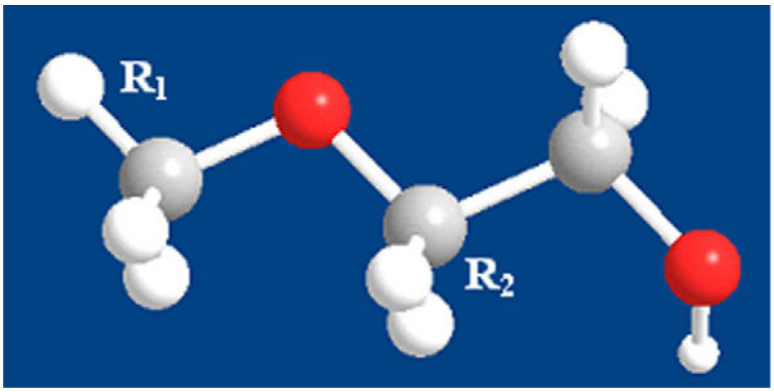

Fig. 1.

Toxicophore elements for the alkoxyethylacetates, alkoxyethanols and their metabolites for QSAR analysis. R1 (alkoxy) moieties R2 (ethanol) moieties $-OCH_3$ 2-methoxy- $-CH_2CH_2OCOCH_3$ ethylacetate group $-OCH_2CH_3$ 2-ethoxy- $-CH_2CH_2OH$ ethanol group $-OCH_2CH_2CH_3$ 2-propoxy- $-CH_2COH$ acetaldehyde group $-OCH_2CH_2CH_2CH_3$ 2-butoxy- $-CH_2COOH$ Acetic acid group.

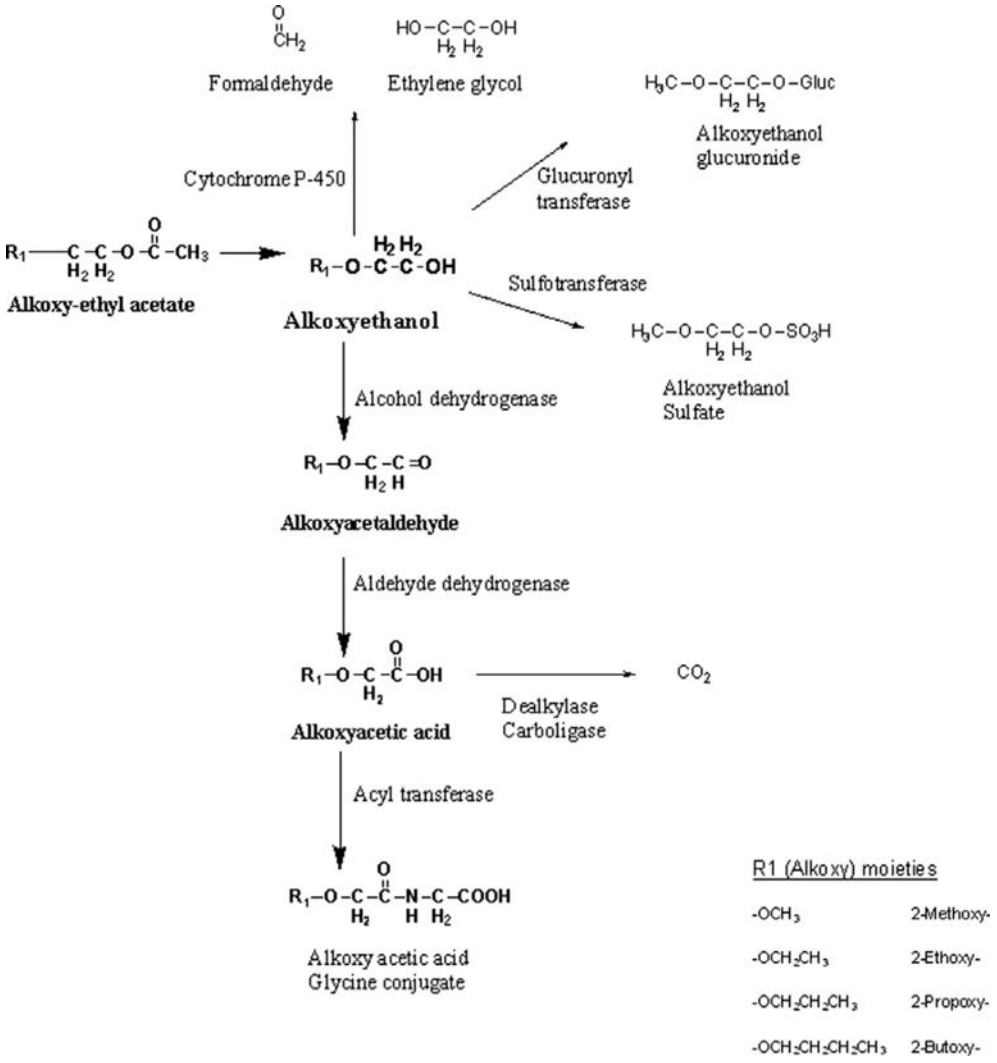

Fig. 2.
Metabolism of alkoxyethanol.

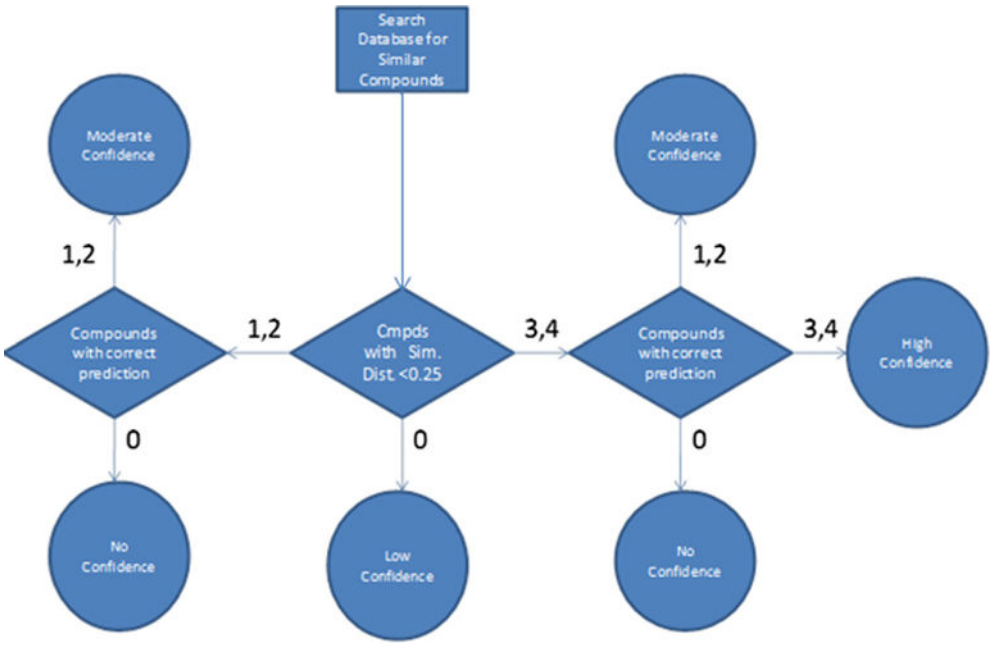

Fig. 3.
The decision matrix for assigning confidence to the model predictions.

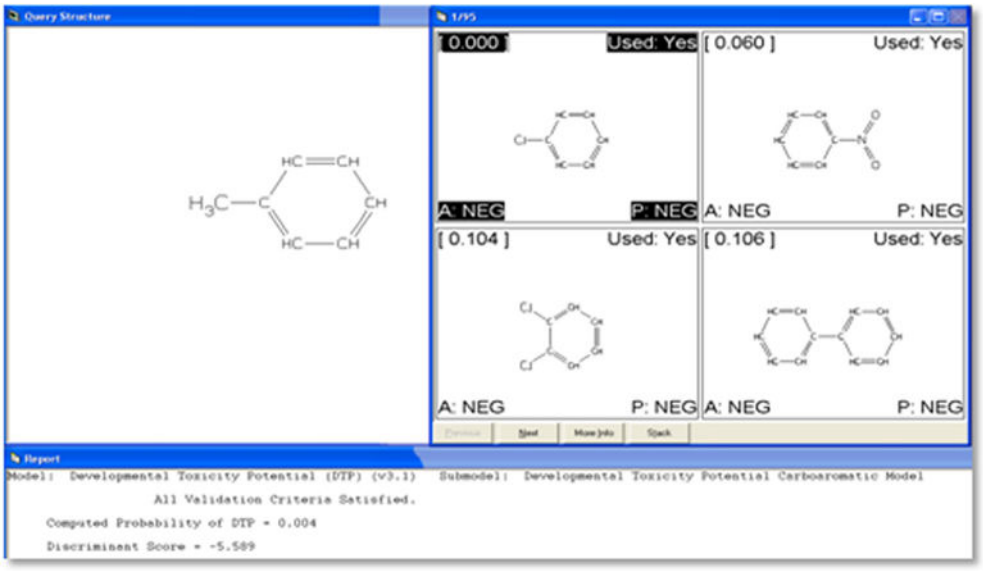

Fig. 4.
QSAR similarity analysis of toluene.

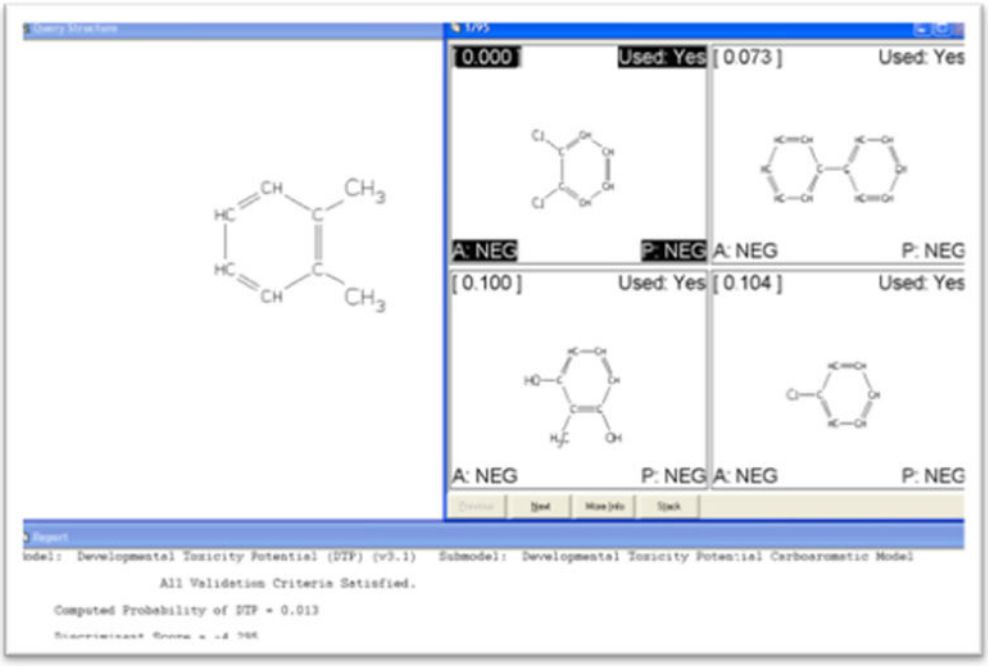

Fig. 5.
QSAR similarity analysis of xylene.

Table 1

BINWOE classification.

Direction of interaction	
=	Additivity
>	More-than-additivity
<	Less-than-additivity
Mechanistic understanding	
I	Direct and unambiguous mechanistic data: The mechanism(s) by which the interactions could occur has been well characterized and leads to an unambiguous interpretation of the direction of the interaction
II	Mechanistic data on related compounds: The mechanism(s) by which the interactions could occur has not been well characterized for the chemicals of concern but structure–activity relationships, either quantitative or informal, can be used to infer the likely mechanisms(s) and the direction of the interaction
III	Inadequate or ambiguous mechanistic data: The mechanism(s) by which the interactions could occur has not been well characterized or information on the mechanism(s) does not clearly indicate the direction that the interaction will have
Toxicological significance	
A	The toxicological significance of the interaction has been directly demonstrated
B	The toxicological significance of the interaction can be inferred or has been demonstrated for related chemicals
C	The toxicological significance of the interaction is unclear

Table 2

The leave-one-out cross-validation study.

Experimental data	Predicted data			Statistics		
	DTP	No DTP	Indeterminate	Total	9	No prediction
DTP	116	17	5	138	87.2%	Sensitivity
No DTP	12	107	4	123	89.9%	Specificity
Total	128	124	9	261	88.5%	Accuracy
Forecast accuracy	90.6%	86.3%	3.45%		96.6%	Applicability

DTP = developmental toxicity potential.

Table 3

Toluene QSAR similarity search analysis.

Ranks	Similarity distances	Developmental toxicity	
		Experimental	Predicted
1. Chlorobenzene	0.000	Negative	Negative
2. Nitrobenzene	0.060	Negative	Negative
3. 2-Dichlorobenzene	0.104	Negative	Negative
4. Diphenyl	0.106	Negative	Negative
5. Aniline	0.193	Negative	Negative
6. *P*-Dichlorobenzene	0.202	Positive	Indetermine
7. 2-Methylresorcinol	0.203	Negative	Negative
8. 2-Phenylphenol	0.229	Negative	Negative
9. 2,4 Dinitrotoluene	0.234	Negative	Negative

Table 4

O-xylene QSAR similarity search analysis.

Ranks	Similarity distances	Developmental toxicity	
		Experimental	Predicted
1. 2-Dichlorobenzene	0.000	Negative	Negative
2. Diphenyl	0.073	Negative	Negative
3. 2-Methylresorcinol	0.100	Negative	Negative
4. Chlorobenzene	0.104	Negative	Negative
5. Nitrobenzene	0.105	Negative	Negative
6. 2-Phenylphenol	0.129	Negative	Negative
7. 2,4 Dinitrotoluene	0.137	Negative	Negative
8. 1,2,3,4,Tetrachlorobenzene	0.193	Positive	Negative
9. *p*-Dichlorobenzene	0.208	Positive	Indetermine
10. Caffeic acid	0.210	Positive	Negative
11. Aniline	0.217	Negative	Negative
12. m-Aminophenol	0.222	Negative	Negative
13. 6-Chloro-4-nitro-2-aminophenol	0.248	Negative	Negative

Table 5

Monte Carlo simulation of acetaldehyde blood concentration (μM) in the US populations.

Population	Year	Mean	Median	99th Percentile	99th Percentile/median
USA	1990 Census	4.2	4.1	7.5	1.83
	2000 Census	4.3	4.1	7.5	1.83
	2010 Census	4.3	4.1	7.7	1.88
	2050 (Projected)	4.3	4.1	13.3	3.23
California	1990 Census	4.6	4.1	22.0	5.37
	2000 Census	4.6	4.1	23.4	5.71
	2010 Census	4.7	4.1	26.0	6.34
Hawaii	1990 Census	6.5	4.2	68.1	16.21
	2000 Census	6.1	4.2	57.1	13.60
	2010 Census	5.9	4.2	53.6	12.77
New Jersey	1990 Census	4.3	4.1	7.5	1.83
	2000 Census	4.3	4.1	7.8	1.9
	2010 Census	4.5	4.1	15.8	3.86
New York	1990 Census	4.3	4.1	7.5	1.83
	2000 Census	4.3	4.1	7.8	1.90
	2010 Census	4.4	4.1	8.7	2.13